

HLA AND IMMUNOGENETICS

Molecular and clinical characterization of HLA – B7 CREG and its comparison with HLA-B27 associated Seronegative Spondarthritis (SSA) Patients.

Year of commencement : 2004.

Year of completion : **On going**

Seronegative spondarthropathies are a group of inflammatory disorders, which clinically involve axial skeleton, sacroilaic & shoulder joints. It has been well established that HLA B27 allele and its novel subtypes are associated with SSA. HLA alleles are highly polymorphic and are known to express in Cross-reactive (CREG) group. HLA B-7 CREG alleles (B7, B27, B22, B40, & B42) have been reported to be involved in Brazilians, Caucasians, African Blacks and Japanese SSA patients.

Till date a total of 624 seronegative spondarthritis patients referred for HLA-B27 typing have been typed for HLA by standard NIH Microlymphocytotoxicity method using specific antisera from commercial and indigenous origin. Three or more sera were used for each specificity. PCR-SSOP Technique has been standardized to know the HLA-B7 subtypes associated in Seronegative spondarthritis patients.

Table1: HLA association in SSA patients and normal controls:

HLA	Pts:n=624 N+	%AF	Ct:n=877 N+	%AF	OR	EF	PF	pValue
B7	133	10.65	178	10.14	1.063			0.631
B22	45	3.6	45	2.56	1.603			0.094
B27	277	22.19	39	2.22	17.15	0.414		4.25E-78
B40	81	6.49	268	15.27	0.338		0.21	1.87E-10

Table 2:HLA- B7 CREG alleles association in B27 negative patients and normal controls:

HLA	Pt:n=347 N+	%AF	Cts:n=838 N+	%AF	OR	EF	PF	value
B7	115	16.57	173	10.32	1.905	0.56		5.02E-06
B22	33	4.75	45	2.68	1.852	0.043		0.0089
B27	0	0	0	0				
B40	53	7.63	268	15.99	0.383		0.19	3.89E-09

Our present data analysis revealed that 1) HLA-B27 is associated strongly with SSA. 2) HLA-B40 is protective in SSA. 3) HLA-B7 allele was significantly associated among HLA-B27 negative patients group. (Table1 and 2). Further when the clinical data were analyzed it showed that HLA-B27 positive patients seem to be different from B27 negative patients. B27 negative patients were found to be relatively of older age (>30yrs: 44% vs. 30%; <30yrs: 56% vs. 70%), and had less family history (16% vs. 27%) when compared to B27 positive patients.

Involvement of HLA antigens in patients with recurrent spontaneous abortion.

Year of Commencement : 2004

Year of Completion : Ongoing

Our immune system protects us from foreign molecules, besides that, it also discriminates between self and non-self components, attacking only foreign molecules. As the fetus is semiallogenic to the maternal immune system, it is recognized as a foreign component. But the maternal immune system protects the developing fetus from such an immunological attack. However, sometimes the foetus is aborted to various etiological factors, which include autoimmune and alloimmune factors, autoimmune factors include autoantibodies such as antinuclear antibody (ANA), antinuetrophilic cytoplasmic antibody (ANCA), antiphospholipid antibody (APA) and antithyroid antibody (ATA). Alloimmune factors include human leukocyte antigens (HLA) and blood group antigens.

Since, HLA alleles control immune response, abnormal immune response due to HLA mismatch between mother, father and the fetus could lead to unsuccessful pregnancy. The present plan has been designed to study HLA antigen in patients with recurrent spontaneous abortion (RSA) with the following objectives:

- To evaluate the development of anti-HLA antibodies in the mother against the paternal tissue and its impact on fetal loss and RSA.
- To study HLA class I antigen expression on mother's lymphocytes and placental tissue and its clinical correlation with RSA.

During the period we have studied a total of 34 couples with 3 or more than three recurrent spontaneous abortion and in control group of 33 couples with one or more successful pregnancy.

In all the above 34 RSA couples (Table : 1) and 33 normal couples (Table 2) anti-HLA antibodies and HLA tissue typing was performed for HLA class I (A&B) antigens following the NIH standard microlymphocytotoxicity test. In short from each individual peripheral blood was collected in heparin anticoagulant and the lymphocytes were separated on histopaque by density gradient centrifugation and the HLA-A and B alleles were identified using specific HLA antisera of commercial and indigenous origin. Each specificity was defined by using three sera with good coefficient of correlation "r" value.

Our initial analysis revealed that the phenotype frequency of HLA A1, A2 and B17 alleles were significantly increased among the RSA patients. Haplotype sharing has been significantly observed among 29 out of the 34 RSA couples (85.29%) in comparison with the 15 out of 33 in control group (45.45%). Haplotype A1B17 and A2B40 significantly increased among RSA couples compared to the fertile

couples. The anti-HLA antibodies were found to be positive in 14.7% of the RSA women while 0.33% among the normal controls.

Table 1: Total RSA patients studied during the period

No.	Sample ID	Age	Sex	RSA	HLA Ab	HLA A	HLA B	HLA A	HLA B
1	8851	27	F	3	Negative	A11	B35	A2	B40
	8852	32	M			A3	B35	A11	B12
2	8859	25	F	3	Negative	A1	B17	A9	B35
	8860	29	M			A1	B17	A19	B44
3	8861	26	F	3	Negative	A2	B27	A9	B12
	8862	31	M			A9	B27	A3	B17
4	8863	25	F	3	Negative	A2	B40	A19	B35
	8864	26	M			A11	B17	A19	B35
5	8866	26	F	3	Negative	A2	B5	A9	B7
	8867	29	M			A9	B35	A11	B7
6	8868	23	F	3	Negative	A2	B8	A10	B5
	8869	30	M			A9	B7	A10	B8
7	8872	32	F	5	Negative	A2	B35	A9	B40
	8873	34	M			A2	B12	A19	B40
8	8874	35	F	3	Negative	A2	B35	A9	B40
	8875	36	M			A2	B12	A3	B40
9	8881	28	F	4	Negative	A1	B17	A9	B5
	8882	31	M			A9	B5	A19	B44
10	8883	26	F	9	Negative	A1	B17	A3	B7
	8884	31	M			A3	B7	A10	B8
11	8888	33	F	5	Negative	A3	B17	A2	B40
	8889	42	M			A3	B17	A19	B35
12	8892	25	F	6	Negative	A1	B17	A19	B22
	8893	28	M			A19	B22	A2	B35
13	8912	31	F	4	Negative	A2	B40	A1	B17
	8913	33	M			A9	B7	A19	B15
14	8919	24	F	3	Negative	A1	B17	A2	B40
	8920	28	M			A2	B40	A19	B35
15	8921	25	F	3	Negative	A1	B17	A2	B40
	8922	30	M			A2	B40	A19	B15
16	8935	30	F	3	Negative	A3	B7	A19	B12
	8936	40	M			A1	B17	A9	B35
17	8943	23	F	3	Negative	A2	B40	A11	B35
	8944	26	M			A2	B40	A3	B7
18	8945	30	F	6	Negative	A1	B17	A19	B27
	8946	38	M			A1	B17	A2	B40
19	8947	26	F	4	Positive	A1	B17	A9	B27
	8948	35	M			A1	B17	A19	B35
20	8985	30	F	3	Negative	A3	B22	A9	B5
	8986	33	M			A1	B17	A2	B40
21	8987	25	F	5	Negative	A3	B12	A19	B5
	8988	31	M			A1	B17	A19	B12
22	8995	25	F	4	Negative	A2	B40	A1	B17

	8996	31	M			A1	B17	A19	B35
23	8997	24	F	3	Negative	A9	B7	A1	B17
	8998	32	M			A2	B40	A1	B17
24	9003	23	F	5	Negative	A3	B5	A1	B17
	9004	26	M			A1	B17	A2	B40
25	9007	26	F	5	Negative	A2	B35	A19	B7
	9008	27	M			A1	B17	A2	B7
26	9036	22	F	4	Positive	A1	B17	A11	B35
	9037	35	M			A2	B40	A19	B12
27	9042	25	F	3	Positive	A3	B12	A19	B22
	9043	30	M			A1	B17	A19	B35
28	9047	30	F	6	Negative	A3	B12	A28	B22
	9048	34	M			A9	B5	A28	B22
29	9049	32	F	3	Negative	A2	B40	A3	B12
	9050	40	M			A1	B17	A3	B12
30	9075	28	F	4	Negative	A1	B17	A19	B56
	9076	30	M			A1	B17	A10	B8
31	9081	32	F	3	Positive	A2	B40	A11	B5
	9082	35	M			A2	B40	A3	B15
32	9083	33	F	3	Negative	A19	B12	A1	B17
	9084	36	M			A19	B12	A3	B35
33	9086	28	F	3	Negative	A1	B17	A19	B5
	9087	30	M			A1	B17	A11	B40
34	9088	20	F	4	Positive	A3	B12	A19	B5
	9089	28	M			A3	B5	A10	B8

Table : 2 Total number of Normal fertile couples types during the period

No.	Sample ID	Age	Sex	HLA Ab	HLA A	HLA B	HLA A	HLA B
1	8890	24	F	Negative	A3	B7	A2	B40
	8891	28	M		A3	B7	A19	B35
2	8916	24	F	Negative	A1	B17	A19	B35
	8917	39	M		A3	B7	A19	B15
3	8962	30	F	Negative	A1	B5	A19	B17
	8963	35	M		A1	B17	A28	B12
4	8970	28	F	Negative	A2	B40	A9	B5
	8971	30	M		A1	B17	A9	B5
5	8972	23	F	Negative	A1	B17	A2	B5
	8973	29	M		A2	B5	A9	B12
6	8974	26	F	Negative	A1	B5	A19	B17
	8975	30	M		A1	B17	A11	B15
7	8976	23	F	Negative	A3	B7	A19	B5
	8977	27	M		A3	B27	A28	B12
8	8981	27	F	Negative	A19	B5	A3	B7

	8982	30	M		A1	B17	A19	B12
9	8983	28	F	Negative	A2	B40	A19	B12
	8984	32	M		A2	B40	A11	B27
10	8991	19	F	Negative	A1	B17	A19	B73
	8992	25	M		A9	B7	A2	B40
11	8993	27	F	Negative	A1	B17	A19	B12
	8994	31	M		A2	B40	A3	B12
12	8999	24	F	Negative	A1	B5	A9	B17
	9000	26	M		A3	B12	A19	B40
13	9001	33	F	Negative	A19	B12	A3	B40
	9002	35	M		A2	B40	A3	B5
14	9013	28	F	Negative	A3	B12	A19	B7
	9014	30	M		A3	B7	A2	B5
15	9016	27	F	Negative	A2	B40	A19	B35
	9017	29	M		A1	B12	A19	B17
16	9018	24	F	Negative	A3	B7	A9	B12
	9018	27	M		A1	B17	A9	B13
17	9020	25	F	Negative	A1	B12	A19	B17
	9021	27	M		A2	B40	A11	B35
18	9022	24	F	Negative	A2	B40	A19	B12
	9023	27	M		A1	B17	A11	B35
19	9024	28	F	Negative	A3	B35	A19	B7
	9025	30	M		A2	B40	A19	B7
20	9028	25	F	Negative	A2	B13	A3	B5
	9029	31	M		A1	B17	A19	B35
21	9034	23	F	Negative	A2	B5	A19	B12
	9035	27	M		A2	B40	A3	B7
22	9040	23	F	Negative	A1	B17	A19	B12
	9041	27	M		A3	B12	A9	B22
23	9044	25	F	Positive	A1	B17	A11	B35
	9045	26	M		A2	B40	A9	B7
24	9052	22	F	Negative	A2	B40	A10	B8
	9053	26	M		A2	B40	A19	B7
25	9054	24	F	Negative	A2	B40	A11	B35
	9055	26	M		A1	B17	A11	B35
26	9056	24	F	Negative	A1	B17	A9	B5
	9057	27	M		A1	B35	A19	B17
27	9058	25	F	Negative	A2	B40	A9	B7
	9059	35	M		A28	B5	A19	B73
28	9060	25	F	Negative	A1	B17	A19	B35
	9061	27	M		A2	B40	A19	B35
29	9062	29	F	Negative	A11	B35	A19	B22
	9063	31	M		A1	B17	A2	B40
30	9064	23	F	Negative	A3	B5	A9	B40
	9065	25	M		A2	B40	A19	B7
31	9068	23	F	Negative	A2	B5	A9	B12
	9069	23	M		A9	B7	A10	B8
32	9072	28	F	Negative	A1	B17	A19	B5

	9073	30	M		A1	B17	A2	B40
33	9108	24	F	Negative	A9	B5	A19	B12
	9109	27	M		A9	B5	A2	B40